biologically realistic environments. Our simulations indicate that water molecules provide important elements in the proton-pumping process. Our findings may form a basis for understanding long-range energy transduction in Complex I, and mechanistic similarities to other redox-driven proton-pumps such as Cytochrome c Oxidase and bacteriorhodopsin.

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S4.P12

Proton and sodium transport by respiratory Complex I devoid of the antiporter-like NuoL subunit

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Complex I is the least understood enzyme of the respiratory chain and its deficiencies have been implicated in several neurodegenerative diseases. It couples the oxidation of NADH and reduction of quinone to charge translocation across the membrane, contributing for the establishment of the membrane potential. Bacterial complex I is general constituted by 14 subunits and has a molecular mass of ~550 kDa, forming an L-shaped structure. These subunits are arranged in two arms, peripheral (NuoB-G and I) and membrane (NuoA, H and J-N). The later arm contains Na^+/H^+ antiporter-like subunits (NuoL, M and N), which are homologous to Na^+/H^+ antiporters (Mrp), suggesting that these subunits may participate in charge translocation. Understanding the energy transduction mechanism is still a major question in complex I research. Crystallographic structural data showed the presence of a ~110 Å long amphipathic helix part of the C-terminal of NuoL subunit, which may function as a coupling element. In this work we investigate the role of NuoL subunit. We used an *Escherichia coli* mutated strain in NuoL from the 'Keio collection' and monitored the ion transport. Proton translocation was studied by quenching of ACMA fluorescence and ^{23}Na NMR spectroscopy was used to investigate sodium transport. We observed that in the absence of the NuoL subunit, although to a small extension proton translocation still occurs, but sodium transport does not. This observation may reflect the role of NuoL in proton and sodium transports or its role in the coupling of this transport machinery. Through a bioinformatic approach we discuss the repercussions on the energy transduction mechanism as well as, the function of the NuoL subunit (particularly its C-terminal extension).

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Modelling the kinetics of complex I

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Modelling the complex I kinetics with biothermokinetic equations. In order to understand the global expression of local mutations in the rate of oxygen consumption or in the ATP production it is useful to have a mathematical model in which the changes in a given respiratory complex (here complex I) are properly modelled. This led us to adapt rate equations containing the essential parameters of enzyme kinetic, maximal velocities and Henri–Michaelis–Menten like-constants (KM and KI) to satisfactorily simulate the experimental complex I kinetics. Finally, we propose a general way to introduce the electrochemical gradient into these equations leading to biothermokinetic rate equations of the respiratory complexes able to simulate the behaviour of the respiratory chain in a large variety of physiological conditions. A stochastic approach of complex I behaviour. A stochastic approach based on the Gillespie algorithm is particularly well adapted to describe the time course of the redox reactions that occur inside the respiratory chain complexes because they involve the motion of single electrons between the individual unique redox centres of a given complex. Using this approach, we showed that the residence time of FMNH• and SQ• (possible producers of ROS) is low (around 4% and between 1.6% and 5% respectively according to the values of the midpoint potentials) and varies according to the direction of the reaction (forwards or backwards). From in silico to in spectro Kinetics of Respiratory Complex I. We compared the simulations of our stochastic model with the experiments performed with a large range of substrates and products. A good fit can be found between the experiments and the prediction of the stochastic approach. A plateau in the kinetics is observed at the highest substrate concentrations, well evidenced in the double reciprocal plots, which is explained by the limiting reaction of quinone reduction as compared with the oxidation of NADH at the other end of the molecule. The set of the seven redox reactions in between the two extreme half redox reactions acts as an electrons buffer.

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Functional differences between two homologous subunits, MrpA and MrpD of the Mrp-type Na^+/H^+ antiporter

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Mrp-type antiporters are monovalent cation/proton antiporters which exchange cytoplasmic Na^+, Li^+ and/or K^+ ions for extracellular H^+. Mrp antiporters consist of six or seven hydrophobic proteins, which are encoded in mrp operon. MrpA and MrpD subunits show similarities to each other and also subunits of a respiratory chain complex I. The NuoL, NuoM and NuoN in the complex I of *Escherichia coli* (NDH-1) are called "antiporter"-like subunits, which are proton-translocating subunits that are coupled to energy from the redox sector. Mutagenesis analyses showed that amino acid residues important for ion transport activities are conserved in all five proteins; MrpA-E140, MrpA-K223, MrpD-E137 and MrpD-K219 [1]. Crystal structure of *E. coli* NDH-1 showed the "antiporter"-like subunits have a common conformational feature, which is an anti-parallel repeat structure composed of 14 transmembrane segments. The region from N-terminus to putative 14th transmembrane helices is well conserved in also MrpA and MrpD. Additionally, NuoL subunit has an amphipathic helix in the carboxyl